Science, medicine, and the future

Nutritional genomics

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The link between diet and health is well established, but renewed interest in which dietary components are biologically active and how they exert their effects is being fuelled by the development of nutritional genomics. Nutritional genomics is the application of high throughput functional genomic technologies in nutrition research. These technologies can be integrated with databases of genomic sequences[1] and inter-individual genetic variability,[2] enabling the process of gene expression to be studied for many thousands of different genes in parallel. Such techniques can facilitate the definition of optimal nutrition at the level of populations, particular groups, and individuals. This in turn should promote the development of food derived treatments and funtionally enhanced foods to improve health.

This review discusses both the science and its potential.

Summary points

- Diet has a substantial impact on chronic disease and health, and functional genomic techniques could allow the bioactivities of food constituents to be defined
- Definition of these activities will allow improvement in health through dietary modification and fortification, novel foods, and "nutraceuticals"
- Challenges lie in the optimal design of nutritional studies and in the effective manipulation of the vast datasets generated
- It is now possible to define gene polymorphisms that predispose individuals to disease and modify nutritional requirements
- Characterisation of such gene polymorphisms will enable targeting of nutritional advice and treatment to "at risk" groups

Methods

This article is based on a review of the literature and our combined personal experience of 19 years working in clinical and molecular nutrition research. It also draws on consensus views for future challenges and opportunities reached at a recent EU funded workshop addressing nutritional genomics, hosted by the Institute of Food Research.

The impact of diet on our health

Evidence that diet is a key environmental factor affecting the incidence of many chronic diseases is overwhelming.[3 4] The precise extent of this contribution is difficult to judge, but a reduction of 35% in the age standardised incidence of cancer in the United States has been proposed to be achievable via "practicable dietary means."[5] Clearly, there is the potential for immense socioeconomic benefit through successful characterisation and exploitation of health promoting factors in foods. The spectrum of the population able to benefit from such research will depend on how the information is used by scientists, the food industry, and policy makers.

How can nutritional genomics help to achieve these goals?

The food we eat contains thousands of biologically active substances, many of which may have the potential to provide substantial health benefits.[3 6] Indeed, several food derived compounds—such as sulphoraphane, curcumin, lycopene, and tea polyphenols—are among the most promising chemopreventive agents being evaluated.[7]

The full extent of biologically active components in our diet is unknown, and our understanding of their mechanisms of action is even more limited. Much of the available data has been derived from in vitro studies with purified compounds in forms and concentrations to which the tissues in our bodies may never be exposed. While this work provides a starting point, more physiologically relevant model systems—including characterisation of the extent and rate of absorption, tissue dispersal, and site specific targeting of metabolically relevant compounds, and comprehensive studies of time and dose effects—are required to interpret the true potential of these constituents. Furthermore, nutrition research has traditionally concentrated on single issues (such as reducing risk of cardiovascular disease or cancer) in "at risk" individuals, whereas what we need to address is the question of

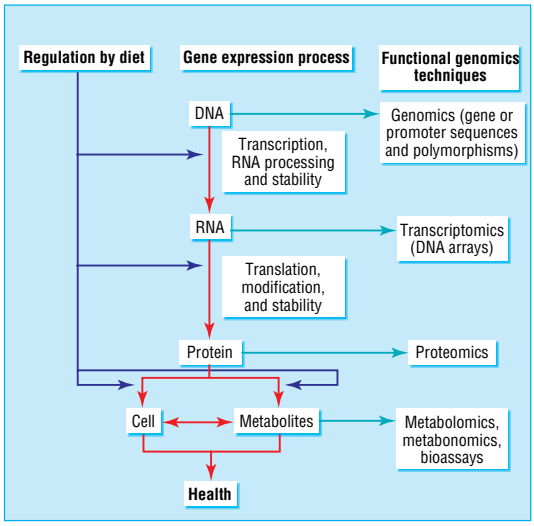

Fig 1 Schematic representation of the steps involved in gene expression (centre), the stages at which diet can modulate these processes (left), and the functional genomics techniques used to analyse each stage (right)

all the possible effects of specific food components in a genetically heterogeneous population. This is especially important for determining unintended risk as well as intended benefit.

Functional genomic technologies

A range of technologies form the practical basis of nutritional genomics (fig 1).[8–10] These are still largely untested in nutritional science, but their potential is underlined by their rapid adoption in disciplines such as pharmaceutical, toxicological, and clinical research. As with these disciplines, the main challenges for nutritional genomics lie in the design of meaningful studies for use of these techniques; the design of studies capable of deciphering the complex interactions between individuals' genetic differences, predisposition to disease, and compound-gene interactions; and the integration and interrogation of the vast data sets that such studies will produce.

Genetic variability

Inter-individual genetic variation is a critical determinant of differences in nutrient requirements. The commonest type of genetic variability is the single nucleotide polymorphism, a single base substitution within the DNA sequence. These occur roughly once every 1000-2000 nucleotides in the human genome.[2] Polymorphism is the "quality of existing in several different forms." It can be the result of genetic predisposition or environmental influence, or a combination of both. In broad terms this is the basis for observed variations in all life forms and individuals. Recent development of extensive genetic polymorphism databases and high throughput genetic screening now make meaningful study of inter-individual variation not only possible but also critical for the future of nutrition and clinical research.

Several genetic polymorphisms of importance to nutrition have been identified (see table).[11–16] For example, common polymorphisms in genes that control folate metabolism have been linked to conditions such as neural tube defects, Down's syndrome, homocystineamia, and cancer.[11 12] If the mechanisms by which these polymorphisms disturb folate metabolism and alter disease risk can be elucidated, it should be possible to develop dietary or therapeutic strategies for "at risk" individuals to redress the balance. Polymorphisms have also been identified in genes involved in lipid metabolism that are important in determining an individual's plasma low density lipoprotein cholesterol concentration, a marker of cardiovascular disease risk.[15]

As more such links between polymorphisms and disease conditions are characterised, the scope for targeting dietary information and recommendations to specific subpopulations will increase. However, before committing ourselves to this approach, it is vital that we consider the logistics and costs of routine genetic screening for many genes, the provision of appropriate counselling, and public attitudes and ethical issues associated with such screening in relation to, say, life insurance and family planning.

Furthermore, resolving the relative roles of gene-gene and gene-environment interactions in polygenic diseases (disorders modulated by multiple genes and polymorphisms within them) is extremely challenging. With osteoporosis, for example, twin and sibling studies suggest that genetic factors are the main determinant of bone mineral density and structure, accounting typically for 50-85% of the phenotypic variance, with environmental factors contributing the rest.[14 17] However,

Glossary of terms

DNA arrays—Analytical tools for measuring the relative amounts of thousands of RNA species within cellular or tissue samples. Sometimes called "transcriptomics," the transcriptome being the complete complement of RNA species produced from the genome of an organism

Genomics—Study of all the nucleotide sequences, including structural genes, regulatory sequences, and non-coding DNA segments, in the chromosomes of an organism

Functional genomics—Application of global (genome-wide or system-wide) experimental approaches to assess gene function

Metabolomics (metabonomics)—Application of system-wide techniques (normally based on nuclear magnetic resonance) for metabolic profiling. Some use the term metabolomics to cover analyses in both simple (cellular) and complex (tissue or whole body) systems. Others distinguish between "metabolomics" studies in simple systems only and "metabonomics" in complex systems

Nutritional genomics—Application of functional genomics approaches to nutrition research

Proteomics—Study of the complete complement of proteins that can be expressed within an organism (a proteome). The commonest practical approach involves comparative analysis of cellular or tissue protein profiles visualised by two dimensional gel electrophoresis and analysed by mass spectrometry of selected protein species

Single nucleotide polymorphism (SNP)—Commonest form of genetic variability in the human genome corresponding to a single nucleotide substitution within a DNA sequence

Examples of known cellular process and known genetic polymorphisms with direct consequences for nutrition

Cellular process	Examples of gene with known polymorphisms	Putative nutritional impact
Folate metabolism[13 14]	Methylene tetrahydrofolate reductase, cystathione β synthase, methionine synthase, glutamate carboxy-peptidase II	Altered risks of neural tube defect, Down's syndrome, cardiovascular disease, and cancer
Iron homoeostasis[15]	Hereditary haemochromatosis linked gene HFE and transferrin receptor	Association with iron requirements, anaemia, and iron overload (haemochromatosis)
Bone health[16]	Vitamin D receptor, oestrogen receptor, type I collagen	Association with bone metabolism, osteoporosis, and mediation of calcium and phosphate translocation
Lipid metabolism[17]	Apolipoproteins (AIV, B, C3, E), low density lipoprotein receptor lipoprotein lipase	Amenable to lipid dietary intervention to modify cardiovascular biomarkers
Immune function[18]	HLA (MHC), tissue necrosis factor α and other cytokines	Predisposition to variable immune responses and susceptibility to food allergies (such as coeliac disease). Possible modulation with dietary lipids (such as polyunsaturated fatty acids) and putative modifications of susceptibility to cancers that are influenced through diet

although some gene polymorphisms have been linked to variations in bone mineral density, these associations are still contentious.[17] It seems likely that several genetic polymorphisms, each making a relatively small contribution, interact to comprise the genetic component associated with osteoporosis. Under such circumstances, candidate gene studies, which seek to find an association between specific gene polymorphisms and markers of disease risk, lack power and may give spurious results. The best strategies for resolving the genetic and environmental contributors to such polygenic disorders are still unclear.[14 17 18]

Fortified and functional foods, dietary supplements, and nutraceuticals

Fortified foods and functional foods are intended to supplement human nutritional needs. Certain foods, such as breakfast cereals, are already routinely fortified with vitamins and minerals, and there is an ever increasing range of functionally enhanced foods with alleged health promoting effects.

Nutraceuticals (or nutriceuticals) are bioactive natural compounds that have health promoting or disease preventing properties. One example is the antihypertensive effect of dietary peptides derived from milk protein, mediated by angiotensin converting enzyme inhibition.[19] Although epidemiological data and preclinical studies are promising, clinical studies of the effect of these milk peptides on human blood pressure have not yet been done.[19] It is crucial that prospective clinical trials incorporate nutrigenomic technologies, especially when comparing these nutritionally derived peptides with synthetically produced angiotensin converting enzyme inhibitors, because responses to the latter possibly depend on gene polymorphism.[20]

People with osteoarthritis might benefit from nutraceuticals such as glucosamine and chondroitin sulphate. A meta-analysis by McAlindon et al and recent findings by Reginster et al suggest that glucosamine sulphate had disease modifying effects and led to symptomatic improvements.[21 22] However, issues of study quality and bias, true efficacy, and toxicity continue to cause uncertainty.[23 24] Further evidence is required from larger high quality clinical trials.

Although some clinical trials of nutraceuticals have shown encouraging results, medical and scientific communities remain sceptical, partly because of concerns about quality control and rigour of scientific testing. Putative nutraceutical compounds are found in a variety of products from the food industry, herbal and dietary supplement producers, pharmaceutical companies, and agribusiness companies. Consequently, the potency and the purity of these agents can vary substantially. Thus, although certain food substances might qualify for health claims if they meet the requirements of the UK Food Standards Agency or the US Food and Drug Administration, they are not as strictly regulated as drugs, which gives rise to concern about routine long term use.

The European Commission has adopted a proposal, arising from the white paper on food safety of 14 January 2000, for a directive on food supplements. This will harmonise the rules for the sale and labelling of vitamins and minerals as dietary supplements. These measures may signal a first step to more comprehensive tightening of legislation, as it is suggested that future amendments could be made to cover products containing other nutrients or ingredients.

Functional genomics techniques are ideal for elucidating the effects of novel functional foods, dietary supplements, and nutraceuticals on global gene expression and cell function without making

assumptions about what to look for in terms of risk. The same approaches are also directly applicable to the assessment of the safety of genetically manipulated foods.

For innovative food products with health benefits to be successful, consumer perception of such products must be positive. Products most likely to succeed are new foods that look and taste good and provide health benefits that consumers understand and desire. The only way to ensure this is to involve consumers in product development. Marketing of new food products with no clear benefit to consumers or that fail to meet expectations will be detrimental for nutrition research and the food industry alike.

Dietary advice and dietary modification

The greatest potential for benefit from dietary modification is likely to be in health maintenance, blocking or slowing the early stages of disease development. However, currently available biomarkers measure parameters that represent steps too far along the disease process (such as subclinical nutritional deficiency or early disease symptoms). Nutritional genomics provides the means to develop molecular biomarkers of early, pivotal changes between health maintenance and disease progression.

Two distinct approaches have been proposed to exploit this opportunity (fig 2). The first focuses on the disease state and tracks back through the mechanism of development to identify the earliest genes involved. These genes might then be used as targets to identify nutritional agents capable of modulating their expression. The second approach starts with the healthy condition and examines the effects of dietary components on global patterns of gene expression without prejudice or expectation. Specific effects on patterns of gene expression would provide the focus to seek links to disease development processes. These approaches need not be mutually exclusive and may be complementary, potentially meeting at the level of the key early genes.

Such work will be complicated by the fact that the natural components of foods we already eat can have both beneficial and adverse effects. These may impinge on quite different health or disease processes and at overlapping doses. For example, moderate to low intake of alcohol is associated with a reduced risk of heart disease but an increased risk of cancer. New approaches for determining maximal benefit and minimal risk will be required to cope with such effects.

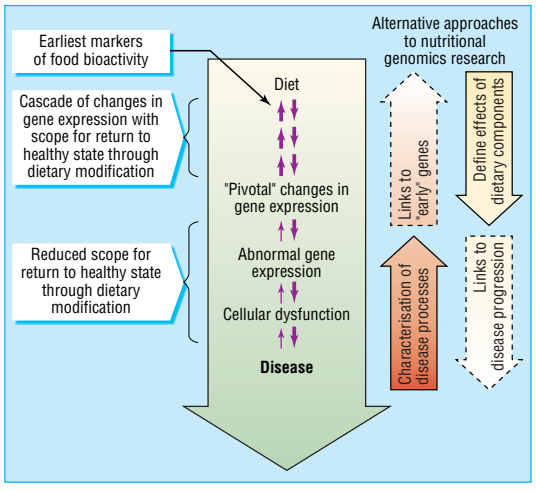

Fig 2 Schematic representation of proposed chronic disease development processes and the alternative nutritional genomics approaches that may be used to characterise them

Additional educational resources

Internet resources

- SNP Consortium (http://snp.cshl.org) provides background and information on single nucleotide polymorphisms for biomedical research
- Resources for microarrays and proteomics (www.functionalgenomics.org.uk, www.microarrays.org, and www.e-proteomics.net/)
- Joint Health Claims Initiative (www.jhci.co.uk) details its code of practice for health claims for food in Britain
- US Food and Drug Administration Center for Food Safety and Applied Nutrition. A food labelling guide (www.cfsan.fda.gov/~dms/flg-6c.html) provides details of the administration's approved list of health claims for foods

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Lesson of the week

Death from tetanus after a pretibial laceration

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It is important to follow the guidelines for treating wounds prone to tetanus

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Clinicians should be familiar with Department of Health guidelines for immunoprophylaxis when wounds through which tetanus can be acquired occur.[1] I report on a patient in whom tetanus immunoprophylaxis did not follow the guidelines.

Case report

A 76 year old woman fell in her garden and sustained a pretibial laceration. Her wound was cleaned and approximated with Steri-strips (3M; Loughborough) at an emergency department. Her status for tetanus immunisation at the time was recorded as "?no previous tetanus injection," and a course of antitetanus treatment was started. However, no immunoglobulin was given.

She returned one week later with a necrotic and malodorous wound. She was unwell and complained of diffuse pains. She was admitted for debridement and split skin grafting.

Her condition worsened. Twenty four hours later she developed the signs and symptoms of tetanus, with increasing jaw stiffness, opisthotonos, and generalised limb spasticity. Cultures from the wound produced a heavy growth of *Clostridium tetanii*. She was transferred to intensive care but died 22 days later.

Discussion

Between 1984 and 1995, 145 cases of tetanus occurred in England and Wales, 75% in people over 45.[1] Tetanus may result from minor wounds as well as from those caused by major trauma and burns.[2]

Prevention is the key to eradicating tetanus. The Department of Health advocates a national immunisation programme and wound immunoprophylaxis (box).[1]

An immunisation programme started in the United Kingdom in 1961. However, anyone over 40 in 2001 has not necessarily been immunised. The uptake of childhood immunisation in some parts of the country may be less than 80%.[3] Background immunisation in the population is poor; in one general practice only 13% of the population was adequately vaccinated.[4] Therefore correct wound assessment and immunoprophylaxis is important.[5 6] This can be divided into two parts. Firstly, the patient should be asked whether they have received a full course of tetanus vaccine and when they last received a booster injection. Secondly, to determine whether the wound is tetanus prone it should be examined and its history ascertained. Correct immunoprophylaxis should follow the published guidelines.[1]

A wound that is prone to tetanus is defined as a wound or burn sustained more than six hours before surgical treatment or with any of the following characteristics: a significant degree of devitalised tissue, a puncture-type wound, contact with soil or manure likely to harbour tetanus organisms, and clinical evidence of sepsis.[1] The only variable that can be altered after wounding is the time from wounding to surgical treatment. This identifies a group of patients, often with relatively minor injuries, whom if treated promptly in the emergency department would never enter this category. This may reduce the requirement for immunoglobulin but will undoubtedly add another pressure to the emergency system.

The management of wounds prone to tetanus in emergency departments can vary. An audit of doctors

Department of Health guidelines for antitetanus prophylaxis of wounds according to immunisation status

Last of three dose course or reinforcing dose within past 10 years
Clean wound—no antitetanus treatment needed
Tetanus prone wound—no antitetanus treatment needed unless risk is thought to be extremely high, for example, contact with manure

Last of three dose course or reinforcing dose more than 10 years previously
Clean wound—reinforcing dose of adsorbed vaccine needed
Tetanus prone wound—give reinforcing dose of adsorbed vaccine and a dose of human tetanus immunoglobulin needed

Not immunised or immunisation status not known with certainty
Clean wound—full three dose course of adsorbed vaccine needed
Tetanus prone wound—full three dose course of vaccine and a dose of immunoglobulin at different site needed